Biological membranes remodel and change shape in processes like endocytosis, exocytosis, hemifusion, and the expansion of fusion pores. Since the length scale of these processes is generally quite small, computational models are needed to resolve the time course of the membrane. Over the past few decades, physicists and mathematicians have developed variational methods for studying time dependent changes in materials like lipid membranes. Building on a model of a single lipidic pore in a vesicle membrane, we direct the variational approach to the study of fusion pores. A fusion pore is a toroidal structure which connects two planar bilayers. The variational approach calculates the time dependent shape of the fusion pore–the precise shape of the pore that minimizes the membrane energy can be calculated using the variational method. The shape of the fusion pore is a solution to the equations of motion which include the surface forces induced by the classical Helfrich energy of the membrane. The energetics involved in the fusion pore expansion are determined as a function of the lipid composition and initial conditions. The model is based on a diffusive interface and director field approach which determines the position and energy of the membrane. The diffusive interface specifies the material properties of lipid and water, accounts for the lipid movements, and is the basis for deriving the equations of motion.

2552-Pos Board B322

Effect of Phosphatidylserine on Asymmetric Membrane Fusion

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While the importance of membrane fusion is long established, the mechanism of membrane fusion is not yet fully understood. Proteins mediate biomembrane fusion, but membrane lipid composition also has a significant role. In order to understand the role of lipid composition in membrane fusion we have studied fusion between fusogenic (PC/PE/SM/CH) and non-fusogenic (DOPC) model membranes. We followed recorded the time courses of lipid mixing, content mixing and content leakage using fluorescence-based assays and then fitted these data to either a two-intermediate or one-intermediate kinetic model. We also examined the effect of negatively charged phosphatidylserine (PS) added to the fusogenic vesicles involved in asymmetric fusion. Our results showed that symmetric fusion between fusogenic membranes is faster and more complete than asymmetric fusion. However, the presence of PS in the fusogenic membrane led to asymmetric fusion being faster than symmetric fusion between PS-containing fusogenic membranes.

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Role of Lipid Tilt in Endocytosis

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The traditional view of endocytosis has focused on the biochemical machinery involved in the process. Here, we focus on the role of mesoscale properties of the plasma membrane in determining the dynamic and equilibrium configurations of the endocytic site. One important lipid-level property is the ability of a lipid to tilt away from the normal to the plane of the membrane. We hypothesize that the lipid tilt degree of freedom is critical for lowering the energy barrier for membrane tubulation and vesicle scission. To test our hypothesis, we have developed a continuum model of lipid tilt, where the membrane energy depends on curvature, tilt and tilt gradient. Our model is novel in that it subsumes previously used theoretical models of membranes of the Helfrich-type models, which emerge as special cases of our model. Using our model, we are able to show that introduction of tilt and spontaneous curvature can lead to asymmetric shapes even in a 1-D formulation. Thus, tilt and tilt gradient are important for symmetry breaking. Our research in now focused on understanding the role of tilt in generating neck constriction at the interface of two different lipid phases and vesicle scission and carrying out simulations in 3-D. We believe that the asymmetry introduced by tilt and tilt gradient are important means to lowering the energy barrier to a membrane fusion or fission pathway. The results from our model will provide insights into the role of mesoscale properties of lipids in governing the macroscale properties of membrane shape and topology.

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Redox Potentials in the Regulation of Viral Fusion

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The acidic interior of endosomes is the trigger for fusion of viruses within this organelle. Whether conditions of endosomal interiors, in addition to low pH, regulate the extent of fusion has not been well explored. The redox potential (RP) within an endosome is regulated by NADPH oxidase and the RP varies according to endosome type. We have investigated whether RP can affect fusion by fusing cells expressing a viral fusion protein to target cells, varying the RP of the external solution. The RP was varied, and clamped, from −150 mV to 0 mV by altering the ratio of Cys/Cyss; cytochrome c was used to establish positive RPs in the external solution. For the class II and class III fusion proteins tested, the more reducing (negative RP) the solution, the greater was the extent of fusion. That is, the extent of fusion varied greatly with RP. The pH-dependence of fusion was, however, independent of RP. For the tested class I fusion proteins, including influenza HA which utilizes the low pH within endosomes, fusion was independent of external redox potentials. To identify the stage(s) of fusion at which redox potentials are consequential, we created a hemifusion intermediate, varying the RP upstream and downstream of the intermediate. The creation of hemifusion was strongly dependent on the RP; the transition from hemifusion to full fusion was less dependent on the RP. Therefore, RPs have strong control on early steps in the fusion process, but only weak control of late steps.

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Acidic Lipid, NBD-PG has Crucial Effect on the Voltage-Dependence of Virus Fusion

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Previously we have shown that cell-cell fusion mediated by Class II and Class III proteins is voltage-dependent, whereas fusion of cells expressing Class I fusion protein is not affected by voltage. Moreover, by testing chimeras consisting of the ectodomain of a Class I protein and the transmembrane domain (TMD) of a Class III protein, we found that TMD was the candidate for the voltage sensor in fusion mediated by Class II and Class III viral proteins.
We hypothesized that, since flip-flop of acidic lipids inherently potential-dependent, incorporation of these lipids into a target membrane should facilitate the fusion at trans-positive membrane potential through affecting the TMD. To test this hypothesis we incorporated a fluorescent acidic lipid (NBD-PG) into target cells membrane and allowed fusion with VSV-G effector cells maintained at negative (−40mV) or positive potential (+40mV) created by the ionophore, SQI-Pr.
We determined that when NPD-PG was present in the target (or effector) cell membranes, the extent of fusion was the same at negative and positive potentials, whereas fusion with untreated cells was inhibited in the presence of ionophore.
Our result show that accumulation of acidic lipids in membranes during fusion directly affects to potential-dependence of fusion.

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pH-Sensitive Pegylated Cationic Lipid-DNA Complexes for Gene Delivery: Transfection Efficiency and Live Cell Imaging Studies

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Gene therapy is a promising new approach for medicine, and able to target a wide variety of diseases. [1]. Cationic liposome (CL)-DNA complexes (also known as lipoplexes) are desirable non-viral gene vectors because of low immunogenicity, ease of scale-up procedures, etc.. To be viable for *in vivo* applications, CL-DNA complexes need to be stable in circulation, which can be achieved by addition of poly-(ethylene glycol) (PEG)-lipids. Unfortunately, PEG-lipids interfere with complex-cell membrane interactions that are vital for particle attachment and uptake and can inhibit membrane fusion required for endosomal escape. The benefit of increased stability from PEG-lipids, thus, comes at the cost of a reduction in transfection efficiency (TE).
To recover the resulting loss in TE, we have designed and synthesized a hydrolysable acid-labile PEG-lipid (HPEG-lipid, PEG MW 2000) which is stable at physiological pH, but is cleaved at low pH. The natural acidification process of endosomes will liberate the PEG from the lipoplexes. We have studied the colloidal stability and transfection efficiency of these HPEG-lipoplexes in mammalian cell cultures. The acid sensitivity and the colloidal stability were characterized by TLC and dynamic light scattering, respectively. The HPEG-lipid is stable at neutral pH for more than 24 h, but degrades completely within 1 h at pH 4, leading to particle aggregation. HPEG-lipoplexes show lower